PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 14/31, A61K 39/085 — A1

(11) International Publication Number: **WO 99/24467**

(43) International Publication Date: 20 May 1999 (20.05.99)

(21) International Application Number: PCT/SE98/02036

(22) International Filing Date: 11 November 1998 (11.11.98)

(30) Priority Data:
9704141-2 12 November 1997 (12.11.97) SE

(71) Applicant *(for all designated States except US)*: SBL VACCIN AB [SE/SE]; Lundagatan 2, S–105 21 Stockholm (SE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: FRYKBERG, Lars [SE/SE]; Stabby Alle 7 C, S–752 29 Uppsala (SE). JACOBSSON, Karin [SE/SE]; Stabby Alle 9 C, S–752 29 Uppsala (SE).

(74) Agents: NILSSON, Brita et al.; AB Stockholms Patentbyrå AB, Zacco & Bruhn (publ), P.O. Box 23101, S–104 35 Stockholm (SE).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: IgG–BINDING PROTEIN FROM *STAPHYLOCOCCUS* AND NUCLEOTIDE SEQUENCE ENCODING THIS PROTEIN

(57) Abstract

A recombinant DNA molecule coding for a protein expressed by a *Staphylococcus aureus* bacterium, comprising the nucleotide sequence SEQ ID NO: 1 or a homologous sequence, or a partial or homologous sequence of SEQ ID NO: 1 coding for a polypeptide fragment comprising at least 15 amino acid residues, is described. Further, a protein expressed by such a bacterium or a polypeptide fragment comprising at least 15 amino acid residues, comprising the amino acid sequence SEQ ID NO: 2 binds IgG and apolipoprotein H. Examples of the polypeptide fragments comprise the SEQ ID NO: 3 through 6. These proteins and polypeptide fragments may be coupled to an inert carrier or matrix. Vectors comprising such a DNA molecule or the corresponding RNA molecule, and antibodies specifically binding to a polypeptide having an amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6, are also disclosed. The DNA or RNA molecules, the vectors and the antibodies mentioned may all be used in different types of vaccines against Staphylococcal infections. Moreover, a method of isolating and/or purifying apoliprotein H from a liquid medium, especially from serum, is described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

IgG–BINDING PROTEIN FROM *STAPHYLOCOCCUS* AND NUCLEOTIDE SEQUENCE ENCODING THIS PROTEIN

The present invention relates to a new protein and a nucleotide sequence encoding said protein. More precisely, the invention relates to a DNA molecule coding for a protein expressed by a bacterium of the genus *Staphylococcus aureus*, said protein and polypeptide fragments of said protein. Vectors comprising the nucleotide sequence coding for the protein, the protein and fragments thereof, and antibodies specifically binding to the protein may all be used for different vaccines against Staphylococcal infections in mammals. The invention also relates to a method of isolating and/or purifying apolipoprotein H from e.g serum with an immobilised protein or polypeptide of the invention.

Background of the invention

Staphylococcus aureus is a pathogen responsible for a wide variety of diseases in humans and animals, including endocarditis, osteomyelitis, wound sepsis and mastitis. The bacterium produces several potential virulence factors such as alpha-, beta-, gamma- and delta-toxins, toxic shock syndrome toxin (TSST), enterotoxins, leucocidin, proteases, coagulase and clumping factor.

It is generally accepted that adhesion to tissues is required for bacterial colonisation to occur. For this purpose staphylococci express surface adhesins, which interact with host matrix proteins such as fibronectin, vitronectin, collagen, laminin and bone sialoprotein. In addition, staphylococci are able to bind several serum proteins, such as IgG, fibronectin, fibrinogen, and thrombospondin, possibly masking the bacteria from the immune system of the host. However, the contribution and importance of each of these binding functions in different infections is still unclear.

The most studied receptor in *S. aureus* is protein A, a cell wall-associated protein, which binds to the Fc- and the Fab-regions of IgG from several species. Protein A in strain 8325-4 consists of five consecutive, highly homologous domains, all with IgG-binding activity, followed by a region anchoring the protein in the cell wall (Uhlén *et al*, 1984). IgG-binding ability is common among clinical strains of *S. aureus* suggesting an important function in pathogenesis. It has been assumed that the IgG-binding capacity is mediated by protein A only.

However, the present inventors recently identified a nucleotide sequence in *S. aureus* strain 8325-4 encoding a polypeptide, clearly distinguishable from protein A, which binds IgG in a non-immune fashion (Jacobsson & Frykberg, 1995). An IgG-binding protein fragment having an amino acid sequence of 84 aa was disclosed. However, the amino acid sequence of the full

length protein and the properties other than the IgG-binding ability of the 84 aa fragment were not known or even suggested. No nucleotide sequence coding for said protein has be disclosed or suggested prior to the present invention.

Diseases caused by Staphylococcal infections are often treated with antibiotics. As is well known in the art, these microorganisms can develop antibiotic resistance. Therefore, the use of vaccines to prevent or contain the spread of infection would be desirable. At present, there is no vaccine on the market that gives full protection. The present invention provides new immunologically active components for the production of vaccines against Staphylococcal infections.

Description of the invention

The present invention is based on cloning and nucleotide sequence determination of a complete gene (*sbi*) encoding a novel IgG-binding protein. The gene encodes a protein of 436 amino acids, denoted protein Sbi, with one IgG-binding domain that exhibits an immunoglobulin-binding specificity similar to protein A and without the typical Gram-positive cell wall anchoring sequence LPXTG (Schneewind *et al*, 1995) suggesting that the protein is not anchored in the cell wall. Analysis of other *S. aureus* strains shows that this gene is not unique for strain 8325-4. For instance, the Sbi-protein is highly expressed in strain Newman 4, which shows that the IgG-binding activity observed in *S. aureus* is not mediated only by protein A. In fact, this *(sbi)* gene is present in all tested strains of *S. aureus*.

Further, it has now been revealed that the Sbi protein of the invention binds apolipoprotein H, a major serum component, in addition to IgG. Hitherto, no bacterial protein binding to apolipoprotein H has been reported. Therefore, neither is this combination of the protein binding to these two serum components previously known. The portion of the protein which binds to IgG is located near the N- terminal of the protein, whereas the middle portion binds to apolipoprotein H. This enables the use of the protein, or an appropriate polypeptide fragment, in immobilised form for the isolation and/or purification of apolipoprotein H.

Thus, one aspect of the present invention is directed to a recombinant DNA molecule coding for a protein expressed by a bacterium of the genus *Staphylococcus aureus*, comprising the nucleotide sequence SEQ ID NO:1, defined in the sequence listing and the claims, or a homologous sequence to SEQ ID NO:1 coding for said protein, or a partial or homologous sequence of the sequence SEQ ID NO:1 coding for a polypeptide fragment of said protein comprising at least 15 amino acid residues.

This recombinant DNA molecule may be inserted into plasmids, phages or phagemides for the expression/production of the protein or protein fragments.

Another aspect of the invention is directed to a protein expressed by a bacterium of the genus *Staphylococcus aureus* or a polypeptide fragment of said protein comprising at least 15 amino acid residues other than the 84 aa fragment at the position 38 - 121, which protein comprises the amino acid sequence SEQ ID NO: 2 , defined in the sequence listing and the claims, or a homologous sequence to the sequence SEQ ID NO: 2 comprising a few mismatches in the amino acid sequence of SEQ ID NO: 2, or polypeptide fragments of said homologous sequence comprising at least 15 amino acid residues.

The disclaimer of the 84 aa fragment at the position 38 - 121 of the SEQ ID NO: 2 is made because, as already mentioned, it has been previously disclosed (Jacobsson & Frykberg, 1995).

It is well known in the art that there may be a few mismatches of amino acids residues in the amino acid sequence of a protein while the protein still retains its major characteristics. The mismatches may be replacements of one or several amino acids, deletions of amino acid residues or truncations of the protein. Such mismatches occur frequently in genetic variations of native proteins. It is believed that up to 15% of the amino acid residues may be replaced in a protein while the protein still retains its major characteristics. The protein of the invention comprises 436 amino acid residues, and therefore up to 66 mismatches would be acceptable. However, preferably there will be less than 20, more preferably less than 10, and most preferably less than 5 mitsmatches in the amino acid sequence of the protein of the invention.

The polypeptide fragments of the protein of the invention should comprise at least 15 amino acid residues to be sure that the fragments are not found in other known proteins. These fragments may be used e.g. as probes, diagnostic antigens, and vaccine components, possibly coupled to carriers.

In an embodiment of this aspect of the invention a polypeptide fragment of the protein according to the invention has the amino acid sequence SEQ ID NO: 3, defined in the sequence listing and the claims. This polypeptide fragment lacks the signal sequence of the SEQ ID NO:1.

In another embodiment a polypeptide fragment of the protein according to the invention has an amino acid sequence SEQ ID NO: 4, defined in the sequence listing and the claims. This polypeptide fragment binds apolipoprotein H.

In yet another embodiment a polypeptide fragment of the protein according to the invention has the amino acid sequence SEQ ID NO: 5, defined in the sequence listing and the claims. This 120 aa polypeptide fragment binds IgG. It was chosen for immunisation purposes, in stead of the known IgG binding 84 aa fragment, since once the whole amino acid sequence was deduced, it became evident there were sequence similarities suggesting two IgG binding domains.

In still another embodiment a polypeptide fragment of the protein according to the invention has the amino acid sequence SEQ ID NO: 6. This polypeptide fragment binds apolipoprotein H, and has been used for isolation and purification of said serum protein.

In a prefered embodiment of this aspect of the invention the protein or polypeptide according to the invention is coupled to an inert carrier or matrix.
The carrier may be e.g. plastic surfaces, such as microplates, beads etc.; organic molecules such as biotin; proteins, such as bovine serum albumin; peptide linkers, polypeptides e.g. resulting in fusion proteins. The matrix may be particles used for chromatographic purposes, such as Sepharose®.

A further aspect of the invention is directed to a vector selected from the group consisting of plasmids, phages or phagemides comprising a nucleotide sequence according to the invention.

These vectors may be used for the production of the proteins or polypeptides of the invention. They may also be used in vaccines.

Yet another aspect of the invention is directed to antibodies specifically binding to a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:4, and SEQ ID NO:6. The specific binding of binding of an antibody to an amino acid sequence of the invention requires e.g an affinity constant of at least 10^7 liters/mole, preferably at least 10^9 liters/mole.

The antibodies of the invention may be monoclonal or polyclonal. They may be used in diagnostic tests, but preferably in vaccines for passive immunization.

Still another aspect of the invention is directed to the use of a protein or polypeptide according to the invention, optionally in immobilised form, as an immunising component in the production of a vaccine against Staphylococcus infections.

Another use aspect of the invention is directed to the use of a vector according to the invention for the production of a vaccine against Staphylococcal infections.

Yet another use aspect of the invention is directed to the use of antibodies according to the invention for the production of a vaccine for the passive immunisation of a mammal against Staphylococcus infections.

An additional aspect of the invention is directed to a vaccine against Staphylococcal infections comprising as an immunising component a protein or polypeptide according to the invention, optionally in immobilised form.

Another vaccine aspect of the invention is directed to a vaccine against Staphylococcal infections comprising a vector according to the invention.

A DNA molecule, or the corresponding RNA derived from the present sequence, as described in claim 1, may be used in a vector for vaccine purposes. Examples of suitable forms of administration include intravenous, percutaneous, and intramuscular administration.

Yet another vaccine aspect of the invention is directed to a vaccine for the passive immunisation of a mammal, especially a human being, against Staphylococcus infections comprising antibodies according to the invention.

One embodiment of the invention comprises the passive immunization of patients with an impaired immune defence or patient awaiting major surgery, such as patients in line for an organ transplantation or awaiting the insertion of a prosthetic device, such as a hip prosthesis or similar major surgical intervention. According to the present invention, a high dose of antibodies against the novel protein can be administered to any patient before or at the time of hospitalisation, in order to prevent Staphylococcus infection.

The vaccines may contain other ingredients selected with regard to the intended aministration rout, and these ingredients are chosen by the vaccine manufacturer in collabaration with pharmacologists. Examples of administration routs include intravenous administration, percutaneous administration, oral and nasal administration.

A further aspect of the invention is directed to a method of prophylactic and/or therapeutic treatment of *Staphylococcus* infections in a mammal comprising administration to said mammal of an immunologically effective amount of a vaccine according to any one of the vaccines of the invention.

Still another aspect of the invention is directed to a method of isolating and/or purifying apolipoprotein H from a liquid medium, especially from serum, comprising chromatographic separation of apolipoprotein H from said liquid medium with an immobilised protein or polypeptide according to the invention as stationary phase.

In a prefered embodiment of the invention column chromatography is used for the isolation/purification of apolipoprotein H from blood serum. The protein or polypeptide of the invention is coupled to e.g. Sepharose® and is used as packing material for the column. The apolipoprotein H-containing serum is brought into contact with the immobilized protein or polypeptide and the apolipoprotein H is adsorbed. Finally, the apolipoprotein H is eluated from the column.

Description of the drawings

Figure 1 shows a) the cloned 3 kb *Pst*I - *Xba*I fragment from *S. aureus* strain 8325-4 containing the *sbi*-gene of the invention (clone pPX1), and b) schematic drawing of the protein Sbi aligned with the peptides encoded by the phagemid clones isolated by panning against IgG. Stars indicate that the clone was isolated several times independently. Ig4 represents the clone isolated earlier (Jacobsson & Frykberg, 1995). S denotes the signal sequence and the shaded bar represents a proline-rich region.

Figures 2 a + 2 b show the complete nucleotide sequence of the *sbi*-gene of the invention from *S. aureus* 8325-4, and the deduced amino acid sequence of the encoded protein. Features of the sequence are indicated as follows: underbar, putative promotor sequences; double underbar, possible ribosomal binding site; overbar, putative transcriptional stop signal (inverted repeat); single vertical arrow indicates the cleavage site of the signal sequence and in bold the repeated proline residues. Also shown in bold is the minimal IgG-binding domain as deduced by shot gun phage display mapping.

Figure 3 shows the specificity of the binding domain of the protein Sbi (clone Ig4) for various IgGs and human IgG3κ, IgG3λ, IgM and IgA. Each column represents a mean value of two independent experiments with the same phage stock. The y-axis shows the number of c.f.u. in 50 µl of the eluate.

Experiments

Analysis of IgG binding activity in *S. aureus* by Western blots usually reveals more than one protein band, which interact with IgG, and it has been assumed that these polypeptides represent breakdown products of protein A. In view of the present results, it is not surprising that protein Sbi has escaped detection. For example, analysis of the expression of the protein Sbi in *S. aureus* has been hampered by the ability of protein A to interact with IgGs from most mammalian species. Furthermore, both proteins migrate similarly in SDS-PAGE and have the same specificity for all tested immunoglobulins. As disclosed in Example 4 below, the

commercially available anti-protein A antibodies cross-react with full-length protein Sbi but not with MAL-SbiΔ (SEQ ID NO:4) (lacking the known IgG-binding domain). Most likely, the cross-reaction is not unique to this source of antibodies and may have contributed to the failure to identify protein Sbi. Now the antibodies directed against the protein Sbi of the invention produced in chicken allows discrimination between expression of protein A and protein Sbi.

The present inventors have cloned and sequenced the *sbi*-gene from *S. aureus* strain 8325-4. The detection of protein Sbi, expressed from its own promoter in *S. epidermidis* and as a MAL-fusion in *E.coli* with HRP-labelled IgG, proves that this gene encodes a second IgG-binding protein. This protein consists of 436 amino acids (SEQ ID NO:2) and contains a signal sequence but lacks the cell wall sorting LPXTG motif, suggesting that the protein is not anchored in the cell-wall. However, in strain Newman 4, which produces high amounts of protein Sbi, no protein Sbi is detected in the culture growth medium (data not shown). Instead, protein Sbi is released from the cell surface by addition of sample buffer (Example 4), in contrast, release of protein A requires treatment with lysostaphin (Example 4). This indicates that protein Sbi is associated to the cell surface by a different mechanism. There are also other examples of cell surface associated proteins that lack the LPXTG motif, such as the *S. aureus* elastin-binding protein (Park et al 1996) and MHC class II analogue (Jönsson et al 1995).

Mapping of protein Sbi by shot-gun phage display strongly suggests that the protein has only one IgG-binding domain with a deduced minimal binding domain of 52 amino acids. Futhermore, expression and analysis of NH_2-terminally truncated Sbi-protein, i.e the C-terminal part of the protein, consisting of aa 143-436 (SEQ ID NO:4), shows that no IgG-binding activity is located in this part of the protein (Example 4). The IgG-binding domain shows a significant homology to the IgG-binding repeats of protein A (Fig. 3).

A phage-stock made from the originally isolated clone, Ig4 (aa 38-121) (Fig. 1b), was used in an analysis of the immunoglobulin species reactivity (Fig. 3). A comparison between data on the specificity of protein A (Boyle, 1990) and the results for clone Ig4 (Fig. 3) shows, that the two proteins exhibit a very similar Ig-binding profile. In addition, a study using one or two binding domains from protein A (clones Ig7 (domain C) and Ig1 (D-A) described by Jacobsson & Frykberg (1995)) gave an Ig-binding profile similar to that of Ig4 (data not shown). The display of protein domain(s) on the phage surface offers a quick and sensitive method for analysing specific binding to other molecules.

Further, the present protein has been shown to bind to another serum protein, apolipoprotein H, also known as β2-glycoprotein I. Hitherto, no bacterial protein binding to

apolipoprotein H has been reported. This finding is unexpected and opens new possibilities to use the protein for isolation/purification of apolipoprotein H for research laboratories and for the production of antibodies against the apolipoprotein H binding portion of the protein. These antibodies may be used as components in vaccines for passive immunisation of mammals against *Staphylococcus* infections.

Examples

Bacterial strains, growth conditions, vectors and helper phage

The bacterial strains used are listed in Table 1. Phage R408 (Promega) was used as the helper phage for production of phage stocks. *E. coli* containing the pUC18 or pMAL-c2 vectors (New England Biolabs) were selected on LA-plates (Luria-Bertani (LB)-broth with 1.5% agar and 50 μg ampicillin ml^{-1}) and grown in LB-broth supplied with 50 μg ampicillin ml^{-1}. *E. coli* containing the phagemid vector pHEN1 (Hoogenboom *et al*, 1991), were grown in the same medium supplemented with 1% glucose (w/v). Staphylococcal strains were grown in Tryptone Soya Broth (TSB) (Oxoid). *S. epidermidis* containing pBR473 was grown in the same medium containing 20 μg ml^{-1} of chloramphenicol.

Table 1. Bacterial strains

Species	Strain	Characteristics and use
E. coli	TG1	F^+ and amber suppressing. Used for construction of the phage library and production of phage stocks.
	MC1061	Used for all other DNA manipulations.
S. aureus	8325-4	NCTC 8325 cured from prophages
	Wood 46	Protein A-negative reference strain
	Newman 4	Spontanteous mutant of strain Newman with enhanced production of fibronectin-binding protein
	Cowan I	NCTC 8350, high level producer of cell-wall- bound protein A.
S. epidermidis 247		

Example 1: Cloning and sequencing of the *sbi* gene encoding an IgG-binding protein

Restriction and modification enzymes were purchased from Promega, Amersham International, or Boehringer Mannheim. Oligonucleotides were synthesized by Scandinavian Gene Synthesis AB or Pharmacia Biotech and are listed in Table 2.

Table 2. Oligonucleotides.

Name	Use	Sequence
Pe	Sequencing of pHEN1 clones	5'- TTG CCT ACG GCA GCC GCT GAA -3'
My	Sequencing of pHEN1 clones	5'- TGC GGC CCC ATT CAG ATC CTC -3'
Olg1	Sequencing of *sbi*	5'- CTC CAT ATA GTA CTT CCT TA-3'
Olg2	Sequencing of *sbi*	5'- GAG ATT GCA TCA TTT GCT GA-3'
Olg3	Sequencing of *sbi*	5'- GTA ACC ATA GTT AAA TGA AT-3'
Olg4	Sequencing of *sbi*	5'- CGA TAA ATC AGC AGC ATA TG-3'
Olg5	Sequencing of *sbi*	5'- CAA TCA CCA CAA ATT GAA AA-3'
Olg6	Sequencing of *sbi*	5'-TGG TGC TTG TAG TGG AAA AG-3'
Olg8	PCR for MAL-E fusions	5'-AGT GGA TCC ACG CAA CAA ACT TCA ACT AAG CA-3'
Olg9	PCR for MAL-E fusions and constr. of *sbi*-probe	5'- AAT GTC GAC AAA CTA GAG AAG ATA TTT TTG A-3'
Olg10	PCR for MAL-E fusions and constr. of *sbi*-probe	5'- TAG GAT CCG TAC AAT CTT CTA AAG CTA AAG A-3'

All DNA manipulations were performed using standard methods (Sambrook *et al*, 1989), except ligations and small scale plasmid preparations, for which the Ready to Go-ligation kit (Pharmacia Biotech) and Wizard™ Miniprep DNA Purification systems (Promega), respectively, were used according to the manufacturers' instructions. Plasmids were introduced into *E. coli* and staphylococci by electrotransformation. Staphylococcal chromosomal DNA was prepared according to Lindberg *et al* (1972).

DNA was sequenced according to the dideoxy chain termination method using the Sequenase® version 2.0 DNA sequencing kit from United States Biochemical. Restriction sites

shown in Fig. 1 were used for construction of subclones used in determination of the nucleotide sequence. One additional clone, pHSBB7, was made (not shown) by *Bal*31 exonuclease digestion from the unique *Hind*III site in the 3′ direction of the gene, and sequenced. Different oligonucleotides were used as primers for determining the sequence of both DNA strands (Table 2). The PC-gene program (Intelligenetics) was used for the handling of the sequences. The EMBL, GeneBank, SWISS-protein and PIR databases were searched for sequence-homologies.

To express the Sbi protein, two constructs were made in the pMAL-c2-vector. Primers Olg8 and 9 were used to PCR-amplify the DNA encoding full-length protein lacking the signal sequence (aa 33-436, SEQ ID NO: 3). Primers Olg9 and 10 were used to PCR-amplify the DNA encoding a truncated version (aa 143-436, SEQ ID NO: 4) lacking also the known IgG-binding domain. The obtained PCR-products were digested with the restriction enzymes *Bam*HI and *Sal*I and ligated into the vector cleaved with the same enzymes and transformed into *E. coli*.

Southern blots and hybridizations were performed according to Sambrook *et al* (1989), using a *Nco*I-*Xho*I fragment, i.e. the complete insert from clone Ig4, (Jacobsson & Frykberg, 1995) ^{32}P-labelled by random priming for detection and cloning of the *sbi*-gene. For detection of the *sbi*-gene in different *S. aureus* strains, the PCR-fragment obtained by using Olg9 and 10 (see above) was ^{32}P-labelled by random-priming. The hybridisation was carried out at 50°C and the washing at 65°C in 0.1 x SSC (20xSSC= 3.0M NaCl and 0.3M Na-citrate) and 0.1 % SDS.

The original clone (Fig. 1b, clone Ig4) expressing an IgG-binding polypeptide was earlier isolated from a shot-gun phage display library made from *S. aureus* strain 8325-4 (Jacobsson & Frykberg, 1995). The insert from this clone was used as a probe for identification and subsequently for cloning of the complete gene from *S. aureus* strain 8325-4. Chromosomal DNA was digested with *Pst*I and *Xba*I, and the DNA fragments were separated by agarose gel electrophoresis followed by blotting onto a nitrocellulose filter. Hybridization with the *Nco*I-*Xho*I fragment, derived from Ig4, showed that the gene of the invention resided on a fragment of approximately 3 kb in size. DNA fragments of this size were purified by agarose gel electrophoresis and cloned into the pUC18 vector. One clone, pPX1, hybridizing with the probe was further characterized by restriction enzyme analysis. Fig. 1 schematically shows the 3 kb *Pst*I-*Xba*I DNA fragment containing the *sbi*-gene and the different restriction enzymes used for subcloning.

Fig. 2 shows 1620 nt of the 3 kb *Pst*I-*Xba*I DNA fragment. The coding sequence starts with a Met at nucleotide position 181 and ends with a stop codon at position 1488 (SEQ ID NO:1), encoding a protein of 436 amino acids (SEQ ID NO: 2), including a typical signal

peptide with a putative cleavage site after amino acid 29. The gene has the normal features associated with a functional gene, putative promotor sequences, a possible ribosomal binding site and an inverted repeat located after the translation termination stop codon. A proline rich sequence, containing eight prolines repeated every fifth amino acid, starts at position 267. Such sequences are normally found within cell wall spanning domains. However, in this case the proline rich region is not followed by the cell wall sorting LPXTG motif (Schneewind *et al*, 1995).

Example 2: Mapping of the IgG-binding domain in protein Sbi

The library was constructed from the cloned *sbi*-gene essentially as described (Jacobsson & Frykberg, 1995). In short, the DNA from clone pPX1 (Fig. 1a) was sonicated and DNA fragments in size of approximately 50 - 300 bp were isolated by preparative gel electrophoresis. The fragments were made blunt-ended with T4 DNA polymerase and ligated into the phagemid pHEN1, previously digested with *Pst*1, made blunt-ended, and dephosphorylated with calf intestine alkaline phosphatase. The ligation was made using 1µg of vector and 1µg of DNA fragments and the mixture was transformed into *E. coli* TG1. The transformants were grown over night in LB supplied with 50 µg ampicillin ml^{-1} and 1% glucose (w/v) and thereafter infected with helper phage R408 at a MOI of 20. After 1 hour the culture was diluted and ampicillin added to a final concentration of 50 µg ml^{-1}. After 5 hours of growth at 37°C, the bacteria were pelletted and the supernatant, containing the phages, was sterile filtered.

The library was affinity selected against human IgG (Kabivitrum) and positive clones were identified using labelled IgG as described (Jacobsson & Frykberg, 1995).

In order to determine the exact position of the IgG-binding domain in protein Sbi a shot-gun phage display library was made from clone pPX1. After panning the library against immobilized human IgG, *E. coli* TG1 cells were infected with the eluted phage and the bacteria were spread on LA-plates containing ampicillin. Amp^R colonies were analysed for binding of HRP-labelled human IgG. Positive clones were isolated and the nucleotide sequences of the different inserts were determined. As seen in Fig. 1 all clones had inserts derived from the same part of *sbi*, suggesting that the encoded protein has only one IgG-binding domain. From the sequences of the binding clones in Fig. 1, the minimal IgG-binding domain is deduced to consist of 52 amino acids.

Example 3: Analysis of the immunoglobulin specificity of protein Sbi

To determine the specificity of protein Sbi, a phage-stock was prepared as described above from clone Ig4, encoding the IgG-binding domain of protein Sbi (Jacobsson & Frykberg,

1995). The stock was diluted to 10^8 phagemid particles ml^{-1} and 100 µl was panned as described (Jacobsson & Frykberg, 1995) against human IgG, IgM, IgA, IgG3κ, IgG3λ as well as IgG from rat, goat, pig, cow, sheep, horse, guinea pig, dog , rabbit and chicken (Sigma), immobilized in microwells at a concentration of 50 µg ml^{-1}. BSA was included as a negative control. The number of bound phagemid particles was determined as c.f.u. after infection of *E. coli* TG1 cells with the phage eluated at pH 2.

To analyse the Ig-binding properties of protein Sbi, clone Ig4 encoding the IgG-binding domain, was used to produce phage-particles displaying the binding domain on the surface. These phages were panned against different immunoglobulins and the number of binding phagemid-particles was determined and used as a measure of the binding ability (Fig. 3).

Example 4: Protein purification and electrophoresis

The MAL-fusion proteins, MAL-Sbi (aa 33- 436, SEQ ID NO:3) and MAL-SbiΔ(aa 143-436, SEQ ID NO:4), were purified from *E. coli* lysates on an amylose-resin according to the manufacturer´s instructions (New England Biolabs). MAL-Sbi was further purified on IgG-Sepharose® according to the manufacturer´s instructions (Pharmacia Biotech).

Antibodies against protein Sbi, developed in chickens, were obtained through Immunsystem AB (Uppsala, Sweden) (four immunizations of 50 µg MAL-SbiΔ-protein). The antibodies were affinity-purified on immobilized MAL-SbiΔ-protein and labelled with Horse-radish peroxidase (HRP) (Boehringer Mannheim). Commercially available HRP-labelled chicken antibodies against protein A were ordered from Immunsystem AB.

To further characterize the protein encoded by the *sbi*-gene the *Pst*1-*Xba*I fragment from pPX1, encoding the complete protein, was made blunt ended and inserted into the *Sma*1 site in the shuttle plasmid pBR473. The construct, pShPX1, was first introduced into the restriction negative *S. aureus* strain 113 and plasmid DNA was prepared from one chloramphenicol resistant colony. The plasmid was then transferred to *S. epidermidis* strain 247, known to be negative in IgG-binding as all coagulase negative staphyolococci, and chloramphenicol resistant colonies were screened for binding of HRP-labeled IgG. All clones analysed had recieved IgG-binding capacity and one of the clones, SePX1, was chosen for further studies. Chromosomal DNA was prepared from this clone, cleaved with *Sna*BI and *Hind*III and analysed by Southern blot hybridization, which showed that the clone contained the *sbi* gene as expected (data not shown).

The bacterial cells in over night-cultures of Newman 4 (2 ml), Cowan I (2 ml) and *S. epidermidis* containing pShPX1 (40 ml) were collected by centrifugation and washed once in

PBS (phosphate buffered saline pH 7.4). Newman 4- and *S. epidermidis*-cells were resuspensed in 40 µl PBS follwed by addition of 40 µl 2x sample-buffer (1x buffer = 125 mM Tris-HCl pH 6.8, 10% glycerol, 5% (v/v) β-mercaptoethanol, 2 % SDS and 0.1% bromophenol blue) and the samples boiled for 2 minutes. To release protein A, the same amount of cells in PBS was treated with lysostaphin (0.1 mg/ml, Sigma) for 5 minutes at 37°C before addition of the sample-buffer and boiling for 2 minutes. The 1µl of the samples were analyzed by SDS-PAGE using the Phast-system (Pharmacia Biotech). Also included were 1/40 µg protein A (Pharmacia Biotech), 1/20 µg MAL-Sbi (SEQ ID NO:3) and 1/20 µg MAL-SbiΔ (SEQ ID NO:4). The proteins were blotted onto nitrocellulose-filters (Schleicher&Schuell) and proteins detected using HRP-labelled affinity purified rabbit anti-chicken (diluted 1/500, Sigma), anti-Sbi (10 µg/ml) or anti-protein A (15 µg/ml). Bound antibodies were detected using 4-chloro-1-naphtol (Serva).

To further characterize the protein encoded by the *sbi*-gene, two clones were made in the pMAL-c2 vector, expressing the mature full length protein (MAL-Sbi, aa 33 - 436, SEQ ID NO:3) and a truncated protein (MAL-SbiΔ, aa 143 - 436, SEQ ID NO:4) lacking the known IgG-binding domain. The purified products were analysed by SDS-PAGE and Western blot together with commercially available protein A, cell surface extract of *S. epidermidis* containing pShX1 and *S. aureus* Newman 4, as well as lysed Newman 4 and Cowan I cells. Three duplicate gels were blotted onto nitrocellulose-filters and the blots developed with HRP-labelled rabbit IgG for detection of protein A and Sbi, anti-protein A and anti-Sbi, respectively.

Western blot analysis of purified proteins shows that antibodies against MAL-SbiΔ (SEQ ID NO:4) recognise both MAL-Sbi (SEQ ID NO:3)and MAL-SbiΔ (SEQ ID NO:4) but not protein A. Commercially available antibodies against protein A (Immunsystem AB, Uppsala) recognise both protein A and MAL-Sbi but not Mal-SbiΔ. This means that the antibodies against protein A recognise the IgG-binding domain in protein Sbi. The antibodies against the polypeptide fragment MAL-SbiΔ of the protein Sbi are specific for the protein Sbi. Western blot analysis of lysates of two different *S. aureus* strains show that the antibodies against MAL-SbiΔ (SEQ ID NO:4) only recognise protein Sbi and no other staphylococcal protein.

Example 5: Occurence of the *sbi*-gene in staphylococcal strains

Chromosomal DNA was isolated from *S. aureus* strains 8325-4, Cowan I, Newman 4 and Wood 46. The DNAs were digested with the enzyme *Hind*III followed by agarose gel

electrophoresis and then blotted onto a nitrocellulose filter. The Southern blot shows that the *sbi*-gene is present in all four *S. aureus* strains tested.

Example 6: Purification of apolipoprotein H

Purified MAL-SbiΔ (SEQ ID NO:4) was immobilised on CNBr-activated Sepharose® (Pharmacia Biotech) according to the manufacturer's instruction. Ten ml rat (ICN), bovine (Life Technologies) or human (Uppsala university hospital) serum was diluted 20 times in PBS and passed over the column. After extensive washing with acetate-buffer pH 5,5, the bound protein was eluted in acetate buffer pH 2.7. The eluate was lyophilised and the protein dissolved in water.

The proteins were analysed by SDS-PAGE and blotted onto nitrocellulose-filters (Schleisher-Schuell) together with commercially available human apolipoprotein H (ICN). The proteins were detected with rabbit anti-human apolipoprotein H antibodies (Chemicon) and HRP-labelled goat anti-rabbit antibodies (Santa Cruz Biotechnology). An identical Western blot was treated with purified MAL-Sbi after which HRP-labelled chicken antibodies against protein Sbi was added. Both filters were developed with 4-chloro-1-naphtol.

These results show that in addition to IgG, protein Sbi binds apolipoprotein H from various mammals and that it can be used for purification of apolipoprotein H.

Example: 7 Purification of apolipoprotein H, and immunsation with protein fragments

Apolipoprotein H from human and bovine sera were also purified on SbiApoB (aa 145-267, SEQ ID NO:6). This domain is used for immunisation in different animals (rat and guinea pig) to investigate if it confers protection against *S. aureus* infections. The recombinant protein SbiIgGB (31-150, SEQ ID NO:5) is also included in this immunisation study.

The Impact™ T7- system from New England Biolabs was used for construction and purification of SbiIgGB (aa 31-150, SEQ ID NO:5) and SbiApoB (aa 145-267, SEQ ID NO: 6). The corresponding parts of the *sbi*-gene was amplified by PCR using the oligonucleotides IgG1pr (5'- CAT GCC ATG GAA AAC ACG CAA CAA ACT TCA- 3'), IgG2pr (5'- TTC TTT AGC TTT AGA AGA -3'), Apo1pr (5'- CAT GCC ATG GAA TCT TCT AAA GCT AAA GAA CGT- 3') and Abobin (5'- TGG CGC CAC TTT CTT TTC AGC-3'). The PCR-products were digested with *Nco*I and treated with T4 polynucleotide kinase and ligated into the pTYB4 vector. The vector had previously been digested with the *Nco*I and *Sma*I and treated with alkaline phosphatase. The constructions were transformed

into BL21(DE3)pLysS-cells and protein was then purified according to the manufacturer´s instructions.

The purified SbiApoB was coupled to a Hitrap® affinity column (Amersham Pharmacia BioTech). Bovine serum (Life Technologies) was applied onto the column. After extensive washing with PBS (phosphate buffered saline) supplied with 0.05% Tween20, bound protein was eluted in 0.1 M glycine pH 3.0. The purified protein was analysed by SDS-PAGE and Western-blotting and was shown to be recognised by rabbit anti-human apolipoprotein H antibodies (Chemicon).

Example: 8 Passive immunisation with protein fragments

As prophylaxis or for treatment of acute infections in a patient , passive immunisation may be effected by administration of antibodies directed against the protein Sbi. These antibodies can be obtained by immunisation of horses with a dose of 50-1000 µg of the polypeptide fragments SEQ ID NO: 4 and/or SEQ ID NO:6 of the protein Sbi, optionally coupled to a carrier, at tree to four separate occasions, followed by purification of the total antibody fraction and/or the specific antibody fraction directed against the non-IgG binding portions of the protein Sbi from serum.

In addition, antibodies can be obtained by immunisation of chickens with 10-100 µg of the polypeptide fragments SEQ ID NO: 4 and/or SEQ ID NO:6 of the protein Sbi, optionally coupled to a carrier proteins per immunisation, at 3-4 separate occasions. The specific antibodies against the non-IgG binding portions of the protein Sbi can then be purified from the eggs and used for passive immunisation. Alternatively, the raw eggs can be consumed to give passive protection.

Finally, antibodies can also be isolated from humans that have recovered from a staphylococcal infection by purification of the total antibody fraction and/or the specific antibody fraction directed against the non-IgG binding portions of the protein Sbi from serum.

Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in the claims appended hereto.

References

Boyle, M. D. P. (1990). *Bacterial Immunoglobulin-Binding Proteins*, vols. 1 and 2, London, Academic Press, Inc. (London), Ltd.

Hoogenboom, H. R., Griffiths, A. D., Johnson, K. S., Chiswell, D. J., Hudson, P. & Winter, G. (1991). Multi-subunit proteins on the surface of filamentous phage: methodologies for displaying antibody (Fab) heavy and light chains. *Nucl Acids Res* **19**, 4133-4137.

Jacobsson, K. & Frykberg, L. (1995). Cloning of ligand-binding domains of bacterial receptors by phage display. *BioTechniques* **18**, 878-885

Jönsson, K., McDevitt, D., Homonylo McGavin, M., Patti, J. M. & Höök, M. (1995). *Staphylococcus aureus* expresses a major histocompatibility complex class II analog. *J. Biol. Chem.* **270**, 21457-21460.

Lindberg, M., Sjöström, J. -E. & Johansson, T. (1972). Transformation of chromosomal and plasmid characters in *Staphylococcus aureus. J Bacteriol* **109**, 844-847.

Park, P.W., Rosenbloom, J., Abrams, W.R., Rosenbloom, J. & Mecham, R.P. (1996). Molecular cloning and expression of the gene for elastin-binding protein (*ebp*S) in *Staphylococcus aureus. J. Biol. Chem.* **271**, 15803-15809.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbour Laboratory.

Schneewind, O., Fowler, A. & Faull, K. F. (1995). Structure of cell wall anchor of surface proteins in *Staphylococcus aureus. Science* **268**, 103-106.

Uhlén, M., Guss, B., Nilsson, B., Gatenbeck, S., Philipson, L. & Lindberg, M. (1984). Complete sequence of the staphylococcal gene encoding protein A, a gene evolved through multiple duplications. *J Biol Chem* **259**, 1695-1702.

Claims

1. Recombinant DNA molecule coding for a protein expressed by a bacterium of the genus *Staphylococcus aureus*, comprising the nucleotide sequence SEQ ID NO:1

```
AGTACTTCCT TACTTAAAAT ACGCTGAATG TTCTGAATTA AACGCTTTTT TACATAGTTA      60
ACACTAGTTA ATCTATTAGT TAACATTAGT TAATAATTAG TTAATTTCCA TTTGTATTCT     120
CATGTGATAA ATTCTAAAAG CATACAATAA ATTTAATATG TAAAAAGAAA GGGAATACAC     180
ATGAAAAATA AATATATCTC GAAGTTGCTA GTTGGGGCAG CAACAATTAC GTTAGCTACA     240
ATGATTTCAA ATGGGGAAGC AAAAGCGAGT GAAAACACGC AACAAACTTC AACTAAGCAC     300
CAAACAACTC AAAACAACTA CGTAACAGAT CAACAAAAAG CTTTTTATCA AGTATTACAT     360
CTAAAAGGTA TCACAGAAGA ACAACGTAAC CAATACATCA AAACATTACG CGAACACCCA     420
GAACGTGCAC AAGAAGTATT CTCTGAATCA CTTAAAGACA GCAAGAACCC AGACCGACGT     480
GTTGCACAAC AAAACGCTTT TTACAATGTT CTTAAAAATG ATAACTTAAC TGAACAAGAA     540
AAAAATAATT ACATTGCACA AATTAAAGAA AACCCTGATA GAAGCCAACA AGTTTGGGTA     600
GAATCAGTAC AATCTTCTAA AGCTAAAGAA CGTCAAAATA TTGAAAATGC GGATAAAGCA     660
ATTAAAGATT TCCAAGATAA CAAAGCACCA CACGATAAAT CAGCAGCATA TGAAGCTAAC     720
TCAAAATTAC CTAAAGATTT ACGTGATAAA AACAACCGCT TTGTAGAAAA AGTTTCAATT     780
GAAAAAGCAA TCGTTCGTCA TGATGAGCGT GTGAAATCAG CAAATGATGC AATCTCAAAA     840
TTAAATGAAA AAGATTCAAT TGAAAACAGA CGTTTAGCAC AACGTGAAGT TAACAAAGCA     900
CCTATGGATG TAAAAGAGCA TTTACAGAAA CAATTAGACG CATTAGTTGC TCAAAAAGAT     960
GCTGAAAAGA AAGTGGCGCC AAAAGTTGAG GCTCCTCAAA TTCAATCACC ACAAATTGAA    1020
AAACCTAAAG TAGAATCACC AAAAGTTGAA GTCCCTCAAA TTCAATCACC AAAAGTTGAG    1080
GTTCCTCAAT CTAAATTATT AGGTTACTAC CAATCATTAA AAGATTCATT TAACTATGGT    1140
TACAAGTATT TAACAGATAC TTATAAAAGC TATAAAGAAA AATATGATAC AGCAAAGTAC    1200
TACTATAATA CGTACTATAA ATACAAAGGT GCGATTGATC AAACAGTATT AACAGTACTA    1260
GGTAGTGGTT CTAAATCTTA CATCCAACCA TTGAAAGTTG ATGATAAAAA CGGCTACTTA    1320
GCTAAATCAT ATGCACAAGT AAGAAACTAT GTAACTGAGT CAATCAATAC TGGTAAAGTA    1380
TTATATACTT TCTACCAAAA CCCAACATTA GTAAAAACAG CTATTAAAGC TCAAGAAACT    1440
GCATCATCAA TCAAAAATAC ATTAAGTAAT TTATTATCAT TCTGGAAATA ATCAATCAAA    1500
AATATCTTCT CTAGTTTTAC ATCATTTTTT AAATAATTTT CGTAACAAAC CGTGATTAAA    1560
AAGAACCGTT GATTCTCAAT CGAATCTACG GTTCTTTTTT CATTTTCCAT CAATTAAATG    1620
```

or a homologous sequence to SEQ ID NO:1 coding for said protein, or a partial or homologous sequence of the sequence SEQ ID NO:1 coding for a polypeptide fragment of said protein comprising at least 15 amino acid residues.

2. Protein expressed by a bacterium of the genus *Staphylococcus aureus* or a polypeptide fragment of said protein comprising at least 15 amino acid residues other than the 84 aa fragment at the position 38 - 121, which protein comprises the amino acid sequence SEQ ID NO: 2

```
 Met Lys Asn Lys Tyr Ile Ser Lys Leu Leu Val Gly Ala Ala Thr Ile
 1               5                  10                  15
 Thr Leu Ala Thr Met Ile Ser Asn Gly Glu Ala Lys Ala Ser Glu Asn
            20                  25                  30
 Thr Gln Gln Thr Ser Thr Lys His Gln Thr Thr Gln Asn Asn Tyr Val
        35                  40                  45
 Thr Asp Gln Gln Lys Ala Phe Tyr Gln Val Leu His Leu Lys Gly Ile
    50                  55                  60
 Thr Glu Glu Gln Arg Asn Gln Tyr Ile Lys Thr Leu Arg Glu His Pro
 65                  70                  75                  80
```

Glu Arg Ala Gln Glu Val Phe Ser Glu Ser Leu Lys Asp Ser Lys Asn
85 90 95
Pro Asp Arg Arg Val Ala Gln Gln Asn Ala Phe Tyr Asn Val Leu Lys
100 105 110
Asn Asp Asn Leu Thr Glu Gln Glu Lys Asn Asn Tyr Ile Ala Gln Ile
115 120 125
Lys Glu Asn Pro Asp Arg Ser Gln Gln Val Trp Val Glu Ser Val Gln
130 135 140
Ser Ser Lys Ala Lys Glu Arg Gln Asn Ile Glu Asn Ala Asp Lys Ala
145 150 155 160
Ile Lys Asp Phe Gln Asp Asn Lys Ala Pro His Asp Lys Ser Ala Ala
165 170 175
Tyr Glu Ala Asn Ser Lys Leu Pro Lys Asp Leu Arg Asp Lys Asn Asn
180 185 190
Arg Phe Val Glu Lys Val Ser Ile Glu Lys Ala Ile Val Arg His Asp
195 200 205
Glu Arg Val Lys Ser Ala Asn Asp Ala Ile Ser Lys Leu Asn Glu Lys
210 215 220
Asp Ser Ile Glu Asn Arg Arg Leu Ala Gln Arg Glu Val Asn Lys Ala
225 230 235 240
Pro Met Asp Val Lys Glu His Leu Gln Lys Gln Leu Asp Ala Leu Val
245 250 255
Ala Gln Lys Asp Ala Glu Lys Lys Val Ala Pro Lys Val Glu Ala Pro
260 265 270
Gln Ile Gln Ser Pro Gln Ile Glu Lys Pro Lys Val Glu Ser Pro Lys
275 280 285
Val Glu Val Pro Gln Ile Gln Ser Pro Lys Val Glu Val Pro Gln Ser
290 295 300
Lys Leu Leu Gly Tyr Tyr Gln Ser Leu Lys Asp Ser Phe Asn Tyr Gly
305 310 315 320
Tyr Lys Tyr Leu Thr Asp Thr Tyr Lys Ser Tyr Lys Glu Lys Tyr Asp
325 330 335
Thr Ala Lys Tyr Tyr Tyr Asn Thr Tyr Tyr Lys Tyr Lys Gly Ala Ile
340 345 350
Asp Gln Thr Val Leu Thr Val Leu Gly Ser Gly Ser Lys Ser Tyr Ile
355 360 365
Gln Pro Leu Lys Val Asp Asp Lys Asn Gly Tyr Leu Ala Lys Ser Tyr
370 375 380
Ala Gln Val Arg Asn Tyr Val Thr Glu Ser Ile Asn Thr Gly Lys Val
385 390 395 400
Leu Tyr Thr Phe Tyr Gln Asn Pro Thr Leu Val Lys Thr Ala Ile Lys
405 410 415
Ala Gln Glu Thr Ala Ser Ser Ile Lys Asn Thr Leu Ser Asn Leu Leu
420 425 430
Ser Phe Trp Lys.
435

or a homologous sequence to the sequence SEQ ID NO: 2 comprising a few mismatches in the amino acid sequence of SEQ ID NO: 2, or polypeptide fragments of said homologous sequence comprising at least 15 amino acid residues.

3. Polypeptide fragment of the protein according to claim 2 having the amino acid sequence SEQ ID NO: 3

Thr Gln Gln Thr Ser Thr Lys His Gln Thr Thr Gln Asn Asn Tyr Val
1 5 10 15
Thr Asp Gln Gln Lys Ala Phe Tyr Gln Val Leu His Leu Lys Gly Ile
20 25 30
Thr Glu Glu Gln Arg Asn Gln Tyr Ile Lys Thr Leu Arg Glu His Pro
35 40 45
Glu Arg Ala Gln Glu Val Phe Ser Glu Ser Leu Lys Asp Ser Lys Asn
50 55 60
Pro Asp Arg Arg Val Ala Gln Gln Asn Ala Phe Tyr Asn Val Leu Lys
65 70 75 80
Asn Asp Asn Leu Thr Glu Gln Glu Lys Asn Asn Tyr Ile Ala Gln Ile
85 90 95
Lys Glu Asn Pro Asp Arg Ser Gln Gln Val Trp Val Glu Ser Val Gln
100 105 110
Ser Ser Lys Ala Lys Glu Arg Gln Asn Ile Glu Asn Ala Asp Lys Ala
115 120 125
Ile Lys Asp Phe Gln Asp Asn Lys Ala Pro His Asp Lys Ser Ala Ala
130 135 140
Tyr Glu Ala Asn Ser Lys Leu Pro Lys Asp Leu Arg Asp Lys Asn Asn
145 150 155 160
Arg Phe Val Glu Lys Val Ser Ile Glu Lys Ala Ile Val Arg His Asp
165 170 175
Glu Arg Val Lys Ser Ala Asn Asp Ala Ile Ser Lys Leu Asn Glu Lys
180 185 190
Asp Ser Ile Glu Asn Arg Arg Leu Ala Gln Arg Glu Val Asn Lys Ala
195 200 205
Pro Met Asp Val Lys Glu His Leu Gln Lys Gln Leu Asp Ala Leu Val
210 215 220
Ala Gln Lys Asp Ala Glu Lys Lys Val Ala Pro Lys Val Glu Ala Pro
225 230 235 240
Gln Ile Gln Ser Pro Gln Ile Glu Lys Pro Lys Val Glu Ser Pro Lys
245 250 255
Val Glu Val Pro Gln Ile Gln Ser Pro Lys Val Glu Val Pro Gln Ser
260 265 270
Lys Leu Leu Gly Tyr Tyr Gln Ser Leu Lys Asp Ser Phe Asn Tyr Gly
275 280 285
Tyr Lys Tyr Leu Thr Asp Thr Tyr Lys Ser Tyr Lys Glu Lys Tyr Asp
290 295 300
Thr Ala Lys Tyr Tyr Tyr Asn Thr Tyr Tyr Lys Tyr Lys Gly Ala Ile
305 310 315 320
Asp Gln Thr Val Leu Thr Val Leu Gly Ser Gly Ser Lys Ser Tyr Ile
325 330 335
Gln Pro Leu Lys Val Asp Asp Lys Asn Gly Tyr Leu Ala Lys Ser Tyr
340 345 350
Ala Gln Val Arg Asn Tyr Val Thr Glu Ser Ile Asn Thr Gly Lys Val
355 360 365
Leu Tyr Thr Phe Tyr Gln Asn Pro Thr Leu Val Lys Thr Ala Ile Lys
370 375 380
Ala Gln Glu Thr Ala Ser Ser Ile Lys Asn Thr Leu Ser Asn Leu Leu
385 390 395 400
Ser Phe Trp Lys.

4. Polypeptide fragment of the protein according to claim 2 having the amino acid sequence SEQ ID NO: 4

```
Val Gln Ser Ser Lys Ala Lys Glu Arg Gln Asn Ile Glu Asn Ala Asp
1               5                  10                  15
Lys Ala Ile Lys Asp Phe Gln Asp Asn Lys Ala Pro His Asp Lys Ser
            20                  25                  30
Ala Ala Tyr Glu Ala Asn Ser Lys Leu Pro Lys Asp Leu Arg Asp Lys
        35                  40                  45
Asn Asn Arg Phe Val Glu Lys Val Ser Ile Glu Lys Ala Ile Val Arg
    50                  55                  60
His Asp Glu Arg Val Lys Ser Ala Asn Asp Ala Ile Ser Lys Leu Asn
65                  70                  75                  80
Glu Lys Asp Ser Ile Glu Asn Arg Arg Leu Ala Gln Arg Glu Val Asn
                85                  90                  95
Lys Ala Pro Met Asp Val Lys Glu His Leu Gln Lys Gln Leu Asp Ala
            100                 105                 110
Leu Val Ala Gln Lys Asp Ala Glu Lys Lys Val Ala Pro Lys Val Glu
        115                 120                 125
Ala Pro Gln Ile Gln Ser Pro Gln Ile Glu Lys Pro Lys Val Glu Ser
    130                 135                 140
Pro Lys Val Glu Val Pro Gln Ile Gln Ser Pro Lys Val Glu Val Pro
145                 150                 155                 160
Gln Ser Lys Leu Leu Gly Tyr Tyr Gln Ser Leu Lys Asp Ser Phe Asn
                165                 170                 175
Tyr Gly Tyr Lys Tyr Leu Thr Asp Thr Tyr Lys Ser Tyr Lys Glu Lys
            180                 185                 190
Tyr Asp Thr Ala Lys Tyr Tyr Tyr Asn Thr Tyr Tyr Lys Tyr Lys Gly
        195                 200                 205
Ala Ile Asp Gln Thr Val Leu Thr Val Leu Gly Ser Gly Ser Lys Ser
    210                 215                 220
Tyr Ile Gln Pro Leu Lys Val Asp Asp Lys Asn Gly Tyr Leu Ala Lys
225                 230                 235                 240
Ser Tyr Ala Gln Val Arg Asn Tyr Val Thr Glu Ser Ile Asn Thr Gly
                245                 250                 255
Lys Val Leu Tyr Thr Phe Tyr Gln Asn Pro Thr Leu Val Lys Thr Ala
            260                 265                 270
Ile Lys Ala Gln Glu Thr Ala Ser Ser Ile Lys Asn Thr Leu Ser Asn
        275                 280                 285
Leu Leu Ser Phe Trp Lys.
    290
```

5. Polypeptide fragment of the protein according to claim 2 having the amino acid sequence SEQ ID NO: 5

```
Glu Asn Thr Gln Gln Thr Ser Thr Lys His Gln Thr Thr Gln Asn Asn
1               5                  10                  15
Tyr Val Thr Asp Gln Gln Lys Ala Phe Tyr Gln Val Leu His Leu Lys
            20                  25                  30
Gly Ile Thr Glu Glu Gln Arg Asn Gln Tyr Ile Lys Thr Leu Arg Glu
        35                  40                  45
His Pro Glu Arg Ala Gln Glu Val Phe Ser Glu Ser Leu Lys Asp Ser
    50                  55                  60
```

```
Lys Asn Pro Asp Arg Arg Val Ala Gln Gln Asn Ala Phe Tyr Asn Val
65                  70                  75                  80

Leu Lys Asn Asp Asn Leu Thr Glu Gln Glu Lys Asn Asn Tyr Ile Ala
                85                  90                  95
Gln Ile Lys Glu Asn Pro Asp Arg Ser Gln Gln Val Trp Val Glu Ser
            100                 105                 110
Val Gln Ser Ser Lys Ala Lys Glu.
        115                 120
```

6. Polypeptide fragment of the protein according to claim 2 having the amino acid sequence SEQ ID NO: 6

```
Ser Ser Lys Ala Lys Glu Arg Gln Asn Ile Glu Asn Ala Asp Lys Ala
1               5                   10                  15
Ile Lys Asp Phe Gln Asp Asn Lys Ala Pro His Asp Lys Ser Ala Ala
            20                  25                  30
Tyr Glu Ala Asn Ser Lys Leu Pro Lys Asp Leu Arg Asp Lys Asn Asn
        35                  40                  45
Arg Phe Val Glu Lys Val Ser Ile Glu Lys Ala Ile Val Arg His Asp
    50                  55                  60
Glu Arg Val Lys Ser Ala Asn Asp Ala Ile Ser Lys Leu Asn Glu Lys
65                  70                  75                  80
Asp Ser Ile Glu Asn Arg Arg Leu Ala Gln Arg Glu Val Asn Lys Ala
                85                  90                  95
Pro Met Asp Val Lys Glu His Leu Gln Lys Gln Leu Asp Ala Leu Val
            100                 105                 110
Ala Gln Lys Asp Ala Glu Lys Lys Val Ala Pro.
        115                 120
```

7. Protein or polypeptide according to any one of claims 2 – 6 coupled to an inert carrier or matrix.

8. Vector selected from the group consisting of plasmids, phages or phagemides comprising a DNA molecule according to claim 1 or the corresponding RNA molecule.

9. Antibodies specifically binding to a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:4, and SEQ ID NO:6.

10. Use of a protein or polypeptide according to any one of claims 2 – 7 as an immunising component in the production of a vaccine against Staphylococcal infections.

11. Use of a vector according to claim 8 for the production of a vaccine against Staphylococcal infections.

12. Use of antibodies according to claim 9 for the production of a vaccine for the passive immunisation of a mammal against Staphylococcal infections.

13. Vaccine against Staphylococcal infections comprising as an immunising component a protein or polypeptide according to any one of claims 2 - 7.

14. Vaccine against Staphylococcal infections comprising a vector according to claim 8.

15. Vaccine for the passive immunisation of a mammal against Staphylococcal infections comprising antibodies according to claim 9.

16. Method of prophylactic and/or therapeutic treatment of Staphylococcal infections in a mammal comprising administration to said mammal of an immunologically effective amount of a vaccine according to any one of claims 12 –14.

17. Method of isolating and/or purifying apolipoprotein H from a liquid medium, especially from serum, comprising chromatographic separation of apolipoprotein H from said liquid medium with an immobilised protein or polypeptide according to claim 7 as the stationary phase.

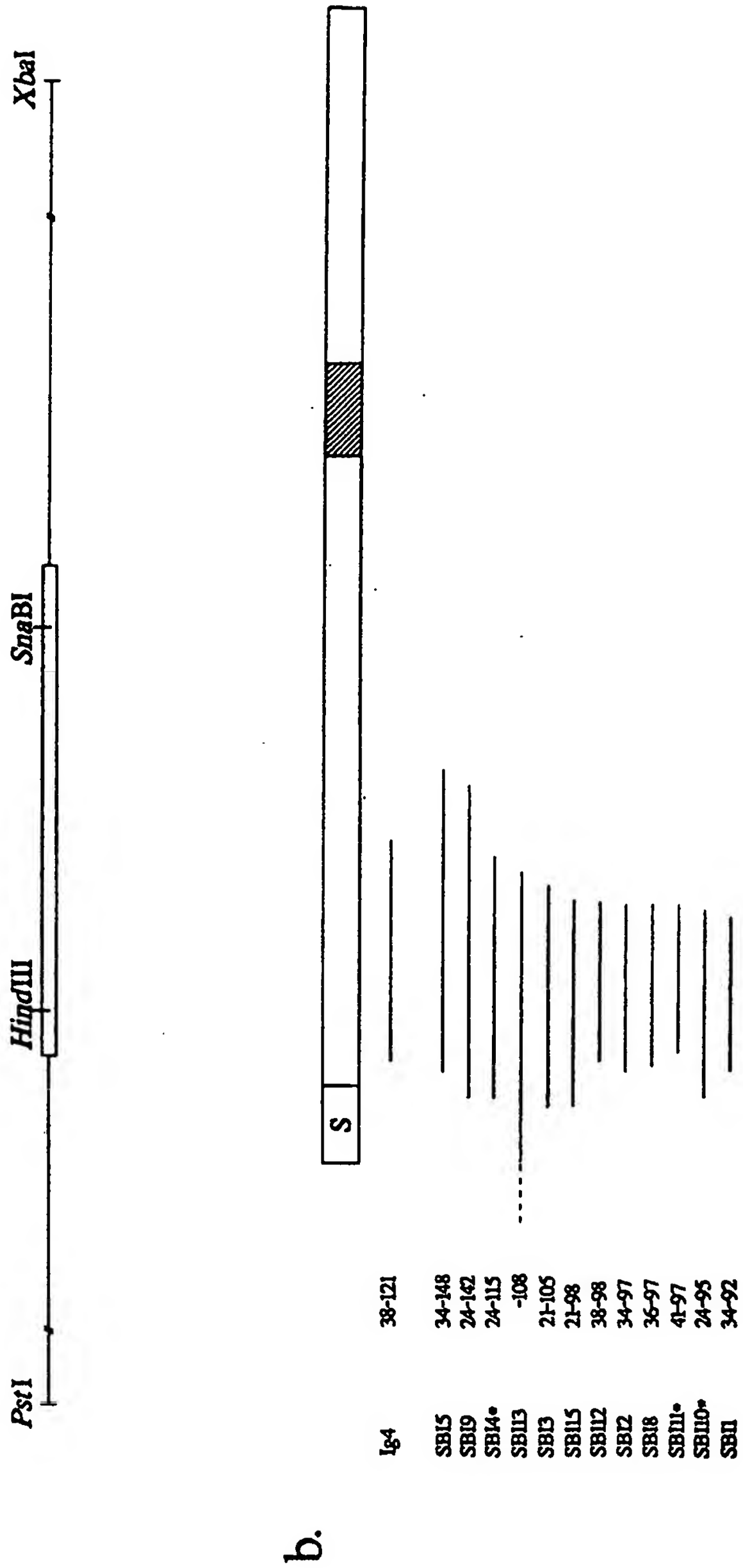
XbaI
SnaBI
HindIII
PstI
S
38-121
34-148
24-142
24-115
-108
21-105
21-98
38-98
34-97
36-97
41-97
24-95
34-92
Lg4
SB15
SB19
SB14*
SB113
SB13
SB115
SB112
SB12
SB18
SB111*
SB110*
SB11
a.
b.

Fig. 1

```
   AGTACTTCCTTACTTAAAATACGCTGAATGTTCTGAATTAAACGCTTTTTTACATAGTTA   60

   ACACTAGTTAATCTATTAGTTAACATTAGTTAATAATTAGTTAATTTCCATTTGTATTCT  120

   CATGTGATAAATTCTAAAAGCATACAATAAATTTAATATGTAAAAAGAAAGGGAATACAC  180

   ATGAAAAATAAATATATCTCGAAGTTGCTAGTTGGGGCAGCAACAATTACGTTAGCTACA  240
 1 M  K  N  K  Y  I  S  K  L  L  V  G  A  A  T  I  T  L  A  T

   ATGATTTCAAATGGGGAAGCAAAAGCGAGTGAAAACACGCAACAAACTTCAACTAAGCAC  300
21 M  I  S  N  G  E  A  K  A  S  E  N  T  Q  Q  T  S  T  K  H

   CAAACAACTCAAAACAACTACGTAACAGATCAACAAAAAGCTTTTTATCAAGTATTACAT  360
41 Q  T  T  Q  N  N  Y  V  T  D  Q  Q  K  A  F  Y  Q  V  L  H

   CTAAAAGGTATCACAGAAGAACAACGTAACCAATACATCAAAACATTACGCGAACACCCA  420
61 L  K  G  I  T  E  E  Q  R  N  Q  Y  I  K  T  L  R  E  H  P

   GAACGTGCACAAGAAGTATTCTCTGAATCACTTAAAGACAGCAAGAACCCAGACCGACGT  480
81 E  R  A  Q  E  V  F  S  E  S  L  K  D  S  K  N  P  D  R  R

   GTTGCACAACAAAACGCTTTTTACAATGTTCTTAAAAATGATAACTTAACTGAACAAGAA  540
101 V  A  Q  Q  N  A  F  Y  N  V  L  K  N  D  N  L  T  E  Q  E

   AAAAATAATTACATTGCACAAATTAAAGAAAACCCTGATAGAAGCCAACAAGTTTGGGTA  600
121 K  N  N  Y  I  A  Q  I  K  E  N  P  D  R  S  Q  Q  V  W  V

   GAATCAGTACAATCTTCTAAAGCTAAAGAACGTCAAAATATTGAAAATGCGGATAAAGCA  660
141 E  S  V  Q  S  S  K  A  K  E  R  Q  N  I  E  N  A  D  K  A

   ATTAAAGATTTCCAAGATAACAAAGCACCACACGATAAATCAGCAGCATATGAAGCTAAC  720
161 I  K  D  F  Q  D  N  K  A  P  H  D  K  S  A  A  Y  E  A  N

   TCAAAATTACCTAAAGATTTACGTGATAAAAACAACCGCTTTGTAGAAAAAGTTTCAATT  780
181 S  K  L  P  K  D  L  R  D  K  N  N  R  F  V  E  K  V  S  I

   GAAAAAGCAATCGTTCGTCATGATGAGCGTGTGAAATCAGCAAATGATGCAATCTCAAAA  840
201 E  K  A  I  V  R  H  D  E  R  V  K  S  A  N  D  A  I  S  K

   TTAAATGAAAAAGATTCAATTGAAAACAGACGTTTAGCACAACGTGAAGTTAACAAAGCA  900
221 L  N  E  K  D  S  I  E  N  R  R  L  A  Q  R  E  V  N  K  A
```

Fig. 2a

```
    CCTATGGATGTAAAAGAGCATTTACAGAAACAATTAGACGCATTAGTTGCTCAAAAAGAT  960
241 P  M  D  V  K  E  H  L  Q  K  Q  L  D  A  L  V  A  Q  K  D

    GCTGAAAAGAAAGTGGCGCCAAAAGTTGAGGCTCCTCAAATTCAATCACCACAAATTGAA 1020
261 A  E  K  K  V  A  P  K  V  E  A  P  Q  I  Q  S  P  Q  I  E

    AAACCTAAAGTAGAATCACCAAAAGTTGAAGTCCCTCAAATTCAATCACCAAAAGTTGAG 1080
281 K  P  K  V  E  S  P  K  V  E  V  P  Q  I  Q  S  P  K  V  E

    GTTCCTCAATCTAAATTATTAGGTTACTACCAATCATTAAAAGATTCATTTAACTATGGT 1140
301 V  P  Q  S  K  L  L  G  Y  Y  Q  S  L  K  D  S  F  N  Y  G

    TACAAGTATTTAACAGATACTTATAAAAGCTATAAAGAAAAATATGATACAGCAAAGTAC 1200
321 Y  K  Y  L  T  D  T  Y  K  S  Y  K  E  K  Y  D  T  A  K  Y

    TACTATAATACGTACTATAAATACAAAGGTGCGATTGATCAAACAGTATTAACAGTACTA 1260
341 Y  Y  N  T  Y  Y  K  Y  K  G  A  I  D  Q  T  V  L  T  V  L

    GGTAGTGGTTCTAAATCTTACATCCAACCATTGAAAGTTGATGATAAAAACGGCTACTTA 1320
361 G  S  G  S  K  S  Y  I  Q  P  L  K  V  D  D  K  N  G  Y  L

    GCTAAATCATATGCACAAGTAAGAAACTATGTAACTGAGTCAATCAATACTGGTAAAGTA 1380
381 A  K  S  Y  A  Q  V  R  N  Y  V  T  E  S  I  N  T  G  K  V

    TTATATACTTTCTACCAAAACCCAACATTAGTAAAAACAGCTATTAAAGCTCAAGAAACT 1440
401 L  Y  T  F  Y  Q  N  P  T  L  V  K  T  A  I  K  A  Q  E  T

    GCATCATCAATCAAAAATACATTAAGTAATTTATTATCATTCTGGAAATAATCAATCAAA 1500
421 A  S  S  I  K  N  T  L  S  N  L  L  S  F  W  K

    AATATCTTCTCTAGTTTTACATCATTTTTTAAATAATTTTCGTAACAAACCGTGATTAAA 1560

    AAGAACCGTTGATTCTCAATCGAATCTACGGTTCTTTTTTCATTTTCCATCAATTAAATG 1620
```

Fig. 2b

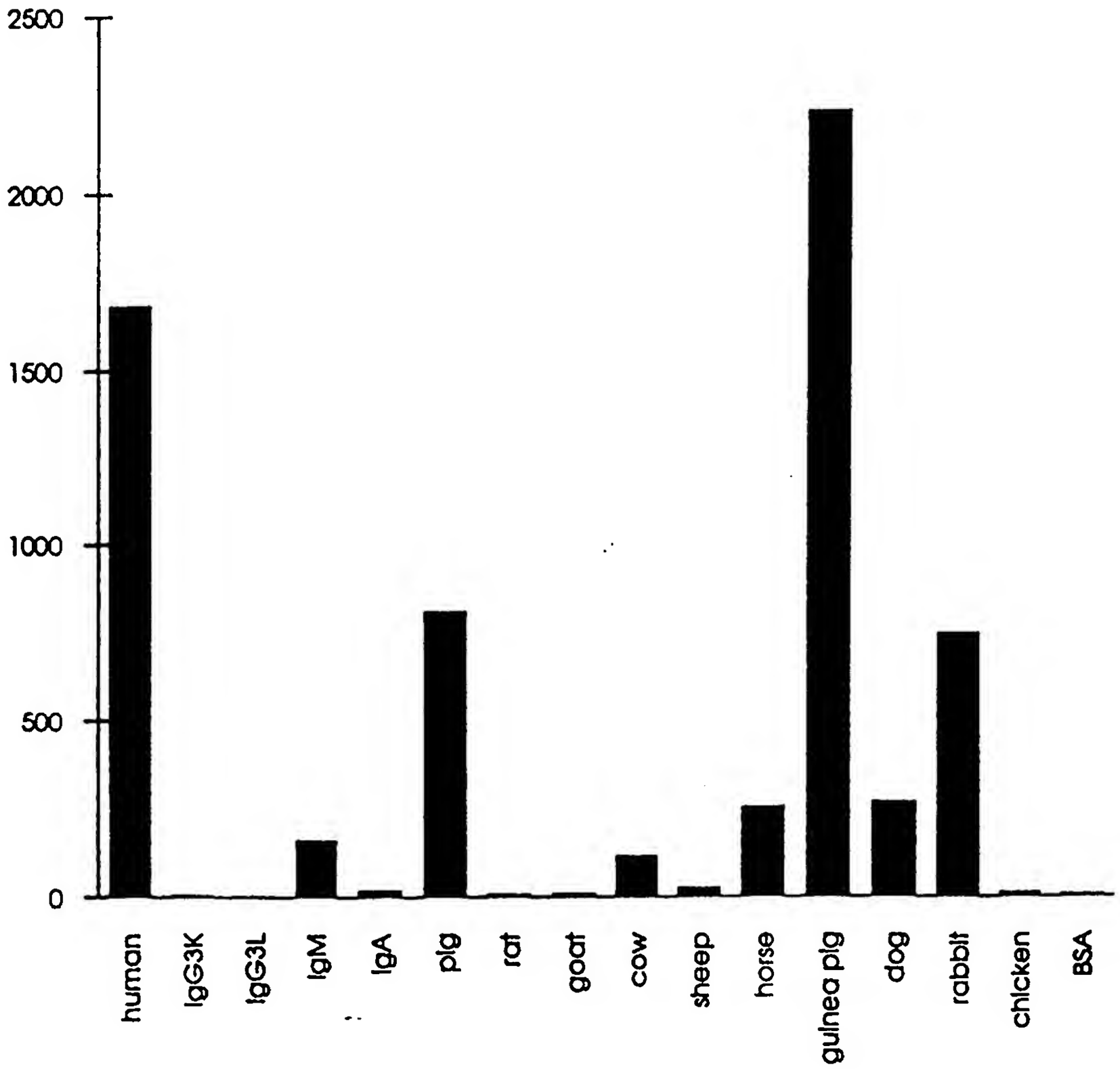
2500
2000
1500
1000
500
0
human
IgG3K
IgG3L
IgM
IgA
pig
rat
goat
cow
sheep
horse
Guinea pig
dog
rabbit
chicken
BSA

Fig. 3

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: SBL Vaccin AB (Publ.)
(B) STREET: Lundagatan 2
(C) CITY: Stockholm
(E) COUNTRY: Sweden
(F) POSTAL CODE (ZIP): 105 21

(ii) TITLE OF INVENTION: New protein and nucleotide sequence encoding said protein

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: SE 9704141-2
(B) FILING DATE: 12-NOV-1997

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1620 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Staphylococcus aureus
(C) INDIVIDUAL ISOLATE: 8325-4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AGTACTTCCT TACTTAAAAT ACGCTGAATG TTCTGAATTA AACGCTTTTT TACATAGTTA 60

ACACTAGTTA ATCTATTAGT TAACATTAGT TAATAATTAG TTAATTTCCA TTTGTATTCT 120

CATGTGATAA ATTCTAAAAG CATACAATAA ATTTAATATG TAAAAAGAAA GGGAATACAC 180

ATGAAAAATA AATATATCTC GAAGTTGCTA GTTGGGGCAG CAACAATTAC GTTAGCTACA 240

ATGATTTCAA ATGGGGAAGC AAAAGCGAGT GAAAACACGC AACAAACTTC AACTAAGCAC 300

CAAACAACTC AAAACAACTA CGTAACAGAT CAACAAAAAG CTTTTTATCA AGTATTACAT 360

CTAAAAGGTA TCACAGAAGA ACAACGTAAC CAATACATCA AAACATTACG CGAACACCCA 420

GAACGTGCAC AAGAAGTATT CTCTGAATCA CTTAAAGACA GCAAGAACCC AGACCGACGT 480

GTTGCACAAC AAAACGCTTT TTACAATGTT CTTAAAAATG ATAACTTAAC TGAACAAGAA 540

AAAAATAATT ACATTGCACA AATTAAAGAA AACCCTGATA GAAGCCAACA AGTTTGGGTA 600

GAATCAGTAC AATCTTCTAA AGCTAAAGAA CGTCAAAATA TTGAAAATGC GGATAAAGCA 660

ATTAAAGATT TCCAAGATAA CAAAGCACCA CACGATAAAT CAGCAGCATA TGAAGCTAAC 720

TCAAAATTAC CTAAAGATTT ACGTGATAAA AACAACCGCT TTGTAGAAAA AGTTTCAATT 780

GAAAAAGCAA TCGTTCGTCA TGATGAGCGT GTGAAATCAG CAAATGATGC AATCTCAAAA 840

TTAAATGAAA AAGATTCAAT TGAAAACAGA CGTTTAGCAC AACGTGAAGT TAACAAAGCA 900

CCTATGGATG TAAAAGAGCA TTTACAGAAA CAATTAGACG CATTAGTTGC TCAAAAAGAT 960

GCTGAAAAGA AAGTGGCGCC AAAAGTTGAG GCTCCTCAAA TTCAATCACC ACAAATTGAA 1020

AAACCTAAAG TAGAATCACC AAAAGTTGAA GTCCCTCAAA TTCAATCACC AAAAGTTGAG 1080

GTTCCTCAAT CTAAATTATT AGGTTACTAC CAATCATTAA AAGATTCATT TAACTATGGT 1140

TACAAGTATT TAACAGATAC TTATAAAAGC TATAAAGAAA AATATGATAC AGCAAAGTAC 1200

TACTATAATA CGTACTATAA ATACAAAGGT GCGATTGATC AAACAGTATT AACAGTACTA 1260

GGTAGTGGTT CTAAATCTTA CATCCAACCA TTGAAAGTTG ATGATAAAAA CGGCTACTTA 1320

GCTAAATCAT ATGCACAAGT AAGAAACTAT GTAACTGAGT CAATCAATAC TGGTAAAGTA 1380

TTATATACTT TCTACCAAAA CCCAACATTA GTAAAAACAG CTATTAAAGC TCAAGAAACT 1440

GCATCATCAA TCAAAAATAC ATTAAGTAAT TTATTATCAT TCTGGAAATA ATCAATCAAA 1500

AATATCTTCT CTAGTTTTAC ATCATTTTTT AAATAATTTT CGTAACAAAC CGTGATTAAA 1560

AAGAACCGTT GATTCTCAAT CGAATCTACG GTTCTTTTTT CATTTTCCAT CAATTAAATG 1620

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 436 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Staphylococcus aureus
(C) INDIVIDUAL ISOLATE: 8325-4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Lys Asn Lys Tyr Ile Ser Lys Leu Leu Val Gly Ala Ala Thr Ile
1 5 10 15

Thr Leu Ala Thr Met Ile Ser Asn Gly Glu Ala Lys Ala Ser Glu Asn
20 25 30

Thr Gln Gln Thr Ser Thr Lys His Gln Thr Thr Gln Asn Asn Tyr Val
35 40 45

Thr Asp Gln Gln Lys Ala Phe Tyr Gln Val Leu His Leu Lys Gly Ile
50 55 60

Thr Glu Glu Gln Arg Asn Gln Tyr Ile Lys Thr Leu Arg Glu His Pro
65 70 75 80

Glu Arg Ala Gln Glu Val Phe Ser Glu Ser Leu Lys Asp Ser Lys Asn
85 90 95

Pro Asp Arg Arg Val Ala Gln Gln Asn Ala Phe Tyr Asn Val Leu Lys
100 105 110

Asn Asp Asn Leu Thr Glu Gln Glu Lys Asn Asn Tyr Ile Ala Gln Ile
115 120 125

Lys Glu Asn Pro Asp Arg Ser Gln Gln Val Trp Val Glu Ser Val Gln
130 135 140

Ser Ser Lys Ala Lys Glu Arg Gln Asn Ile Glu Asn Ala Asp Lys Ala
145 150 155 160

Ile Lys Asp Phe Gln Asp Asn Lys Ala Pro His Asp Lys Ser Ala Ala
165 170 175

Tyr Glu Ala Asn Ser Lys Leu Pro Lys Asp Leu Arg Asp Lys Asn Asn
180 185 190

Arg Phe Val Glu Lys Val Ser Ile Glu Lys Ala Ile Val Arg His Asp
195 200 205

Glu Arg Val Lys Ser Ala Asn Asp Ala Ile Ser Lys Leu Asn Glu Lys
210 215 220

Asp Ser Ile Glu Asn Arg Arg Leu Ala Gln Arg Glu Val Asn Lys Ala
225 230 235 240

Pro Met Asp Val Lys Glu His Leu Gln Lys Gln Leu Asp Ala Leu Val
245 250 255

Ala Gln Lys Asp Ala Glu Lys Lys Val Ala Pro Lys Val Glu Ala Pro
260 265 270

Gln Ile Gln Ser Pro Gln Ile Glu Lys Pro Lys Val Glu Ser Pro Lys
275 280 285

Val Glu Val Pro Gln Ile Gln Ser Pro Lys Val Glu Val Pro Gln Ser
290 295 300

```
Lys Leu Leu Gly Tyr Tyr Gln Ser Leu Lys Asp Ser Phe Asn Tyr Gly
305                 310                 315                 320

Tyr Lys Tyr Leu Thr Asp Thr Tyr Lys Ser Tyr Lys Glu Lys Tyr Asp
                325                 330                 335

Thr Ala Lys Tyr Tyr Tyr Asn Thr Tyr Tyr Lys Tyr Lys Gly Ala Ile
            340                 345                 350

Asp Gln Thr Val Leu Thr Val Leu Gly Ser Gly Ser Lys Ser Tyr Ile
        355                 360                 365

Gln Pro Leu Lys Val Asp Asp Lys Asn Gly Tyr Leu Ala Lys Ser Tyr
    370                 375                 380

Ala Gln Val Arg Asn Tyr Val Thr Glu Ser Ile Asn Thr Gly Lys Val
385                 390                 395                 400

Leu Tyr Thr Phe Tyr Gln Asn Pro Thr Leu Val Lys Thr Ala Ile Lys
                405                 410                 415

Ala Gln Glu Thr Ala Ser Ser Ile Lys Asn Thr Leu Ser Asn Leu Leu
            420                 425                 430

Ser Phe Trp Lys
        435
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 404 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: C-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Staphylococcus aureus
(C) INDIVIDUAL ISOLATE: 8325-4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
Thr Gln Gln Thr Ser Thr Lys His Gln Thr Thr Gln Asn Asn Tyr Val
1               5                   10                  15

Thr Asp Gln Gln Lys Ala Phe Tyr Gln Val Leu His Leu Lys Gly Ile
            20                  25                  30

Thr Glu Glu Gln Arg Asn Gln Tyr Ile Lys Thr Leu Arg Glu His Pro
        35                  40                  45

Glu Arg Ala Gln Glu Val Phe Ser Glu Ser Leu Lys Asp Ser Lys Asn
    50                  55                  60

Pro Asp Arg Arg Val Ala Gln Gln Asn Ala Phe Tyr Asn Val Leu Lys
65                  70                  75                  80
```

```
Asn Asp Asn Leu Thr Glu Gln Glu Lys Asn Asn Tyr Ile Ala Gln Ile
                85                  90                  95

Lys Glu Asn Pro Asp Arg Ser Gln Gln Val Trp Val Glu Ser Val Gln
            100                 105                 110

Ser Ser Lys Ala Lys Glu Arg Gln Asn Ile Glu Asn Ala Asp Lys Ala
        115                 120                 125

Ile Lys Asp Phe Gln Asp Asn Lys Ala Pro His Asp Lys Ser Ala Ala
    130                 135                 140

Tyr Glu Ala Asn Ser Lys Leu Pro Lys Asp Leu Arg Asp Lys Asn Asn
145                 150                 155                 160

Arg Phe Val Glu Lys Val Ser Ile Glu Lys Ala Ile Val Arg His Asp
                165                 170                 175

Glu Arg Val Lys Ser Ala Asn Asp Ala Ile Ser Lys Leu Asn Glu Lys
            180                 185                 190

Asp Ser Ile Glu Asn Arg Arg Leu Ala Gln Arg Glu Val Asn Lys Ala
        195                 200                 205

Pro Met Asp Val Lys Glu His Leu Gln Lys Gln Leu Asp Ala Leu Val
    210                 215                 220

Ala Gln Lys Asp Ala Glu Lys Lys Val Ala Pro Lys Val Glu Ala Pro
225                 230                 235                 240

Gln Ile Gln Ser Pro Gln Ile Glu Lys Pro Lys Val Glu Ser Pro Lys
                245                 250                 255

Val Glu Val Pro Gln Ile Gln Ser Pro Lys Val Glu Val Pro Gln Ser
            260                 265                 270

Lys Leu Leu Gly Tyr Tyr Gln Ser Leu Lys Asp Ser Phe Asn Tyr Gly
        275                 280                 285

Tyr Lys Tyr Leu Thr Asp Thr Tyr Lys Ser Tyr Lys Glu Lys Tyr Asp
    290                 295                 300

Thr Ala Lys Tyr Tyr Tyr Asn Thr Tyr Tyr Lys Tyr Lys Gly Ala Ile
305                 310                 315                 320

Asp Gln Thr Val Leu Thr Val Leu Gly Ser Gly Ser Lys Ser Tyr Ile
                325                 330                 335

Gln Pro Leu Lys Val Asp Asp Lys Asn Gly Tyr Leu Ala Lys Ser Tyr
            340                 345                 350

Ala Gln Val Arg Asn Tyr Val Thr Glu Ser Ile Asn Thr Gly Lys Val
        355                 360                 365

Leu Tyr Thr Phe Tyr Gln Asn Pro Thr Leu Val Lys Thr Ala Ile Lys
    370                 375                 380

Ala Gln Glu Thr Ala Ser Ser Ile Lys Asn Thr Leu Ser Asn Leu Leu
385                 390                 395                 400
```

Ser Phe Trp Lys

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 294 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: C-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Staphylococcus aureus
(C) INDIVIDUAL ISOLATE: 8325-4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Val Gln Ser Ser Lys Ala Lys Glu Arg Gln Asn Ile Glu Asn Ala Asp
1 5 10 15

Lys Ala Ile Lys Asp Phe Gln Asp Asn Lys Ala Pro His Asp Lys Ser
20 25 30

Ala Ala Tyr Glu Ala Asn Ser Lys Leu Pro Lys Asp Leu Arg Asp Lys
35 40 45

Asn Asn Arg Phe Val Glu Lys Val Ser Ile Glu Lys Ala Ile Val Arg
50 55 60

His Asp Glu Arg Val Lys Ser Ala Asn Asp Ala Ile Ser Lys Leu Asn
65 70 75 80

Glu Lys Asp Ser Ile Glu Asn Arg Arg Leu Ala Gln Arg Glu Val Asn
85 90 95

Lys Ala Pro Met Asp Val Lys Glu His Leu Gln Lys Gln Leu Asp Ala
100 105 110

Leu Val Ala Gln Lys Asp Ala Glu Lys Lys Val Ala Pro Lys Val Glu
115 120 125

Ala Pro Gln Ile Gln Ser Pro Gln Ile Glu Lys Pro Lys Val Glu Ser
130 135 140

Pro Lys Val Glu Val Pro Gln Ile Gln Ser Pro Lys Val Glu Val Pro
145 150 155 160

Gln Ser Lys Leu Leu Gly Tyr Tyr Gln Ser Leu Lys Asp Ser Phe Asn
165 170 175

Tyr Gly Tyr Lys Tyr Leu Thr Asp Thr Tyr Lys Ser Tyr Lys Glu Lys
180 185 190

Tyr Asp Thr Ala Lys Tyr Tyr Tyr Asn Thr Tyr Tyr Lys Tyr Lys Gly

195 200 205

Ala Ile Asp Gln Thr Val Leu Thr Val Leu Gly Ser Gly Ser Lys Ser
210 215 220

Tyr Ile Gln Pro Leu Lys Val Asp Asp Lys Asn Gly Tyr Leu Ala Lys
225 230 235 240

Ser Tyr Ala Gln Val Arg Asn Tyr Val Thr Glu Ser Ile Asn Thr Gly
245 250 255

Lys Val Leu Tyr Thr Phe Tyr Gln Asn Pro Thr Leu Val Lys Thr Ala
260 265 270

Ile Lys Ala Gln Glu Thr Ala Ser Ser Ile Lys Asn Thr Leu Ser Asn
275 280 285

Leu Leu Ser Phe Trp Lys
290

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Staphylococcus aureus
(C) INDIVIDUAL ISOLATE: 8325-4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Glu Asn Thr Gln Gln Thr Ser Thr Lys His Gln Thr Thr Gln Asn Asn
1 5 10 15

Tyr Val Thr Asp Gln Gln Lys Ala Phe Tyr Gln Val Leu His Leu Lys
20 25 30

Gly Ile Thr Glu Glu Gln Arg Asn Gln Tyr Ile Lys Thr Leu Arg Glu
35 40 45

His Pro Glu Arg Ala Gln Glu Val Phe Ser Glu Ser Leu Lys Asp Ser
50 55 60

Lys Asn Pro Asp Arg Arg Val Ala Gln Gln Asn Ala Phe Tyr Asn Val
65 70 75 80

Leu Lys Asn Asp Asn Leu Thr Glu Gln Glu Lys Asn Asn Tyr Ile Ala
85 90 95

Gln Ile Lys Glu Asn Pro Asp Arg Ser Gln Gln Val Trp Val Glu Ser
100 105 110

Val Gln Ser Ser Lys Ala Lys Glu
115 120

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/02036

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/31, A61K 39/085
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Biotechniques, Volume 18, No 5, 1995, Karin Jacobsson et al, "Cloning of Ligand-Binding Domains of Bacterial Receptors by Phage Display", page 3 - page 9, figure 2, and the whole document ---	1-3,5,7-8, 10-14
X	Databas Swissprot:Spa2_Staau, accession no. P38507, Shuttleworth H.L. et al: "Immunoglobulin G binding protein A precursor (protein A)"; & Gene 58:283-295 (1987) ---	1
P,X	Microbiology, Volume 144, 1998, Lihong Zhang et al, "A second IgG-binding protein in Staphylococcus aureus" page 985 - page 991 --- --------	1-15,17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 February 1999	28 -02- 1999
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Patrick Andersson Telephone No. +46 8 782 25 00